Predator-prey space use in response to chemical cues of predation

Undergraduate Research Thesis

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Abstract

Interactions between predators and prey are a major component of ecosystems and have the potential to shape ecosystem dynamics. As predators and prey move together spatially and temporally throughout their habitat, each makes decisions to increase its own fitness. To make optimal movement decisions, individuals must accurately interpret their surroundings using available information. Two chemical cues are important for predator-prey interactions: predator kairomones and prey alarm cues, the chemical cue components of a typical predation event. How organisms use available information to make movement decisions and how their space use differs after chemical cue exposure is not yet fully understood. We measured space use within a system of predatory dragonfly larvae (*Anax junius*) and green frog tadpoles (*Rana* [=*Lithobates*] *clamitans*) exposed to chemical cues. To determine how predators and prey interpret predation events and make movement decisions, we conducted experiments using the components of a predation event: kairomones and alarm cues. We hypothesized that tadpoles would respond more strongly when exposed to a combination of chemical cues and that *Anax* would attempt to match prey distributions to increase its predation success rate. We found that tadpoles only responded with antipredator behaviors (i.e., spatially move away from perceived predation risk) when exposed to both *Anax* kairomones and conspecific alarm cues and that predatory *Anax* do not differ in their space use after chemical cue exposure. Our results suggest that tadpole prey minimize potentially costly antipredator behavior by selectively responding to environmental information and that predators are behaviorally managing prey fear. Our research has shed insight into how predators and prey use different chemical cues when making movement decisions. Our results can be applied to aquatic and terrestrial systems where predator-prey species rely on chemical cues.

Introduction

Predators can negatively impact their prey in two ways: through direct consumption and by inducing phenotypic changes (non-consumptive effects; Werner and Peacor, 2003). Interactions between predators and prey are a major component of ecosystems and have the potential to shape population, community, and ecosystem dynamics (Lima and Dill, 1990). The importance of these interactions is frequency-dependent, which their importance increasing with increasing encounter rates between predators and prey (Krivan, 1997; van Baalen and Sabelis, 1993; van Baalen and Sabelis, 1999). How predators and prey interact is thus a product of how individuals distribute themselves spatially and temporally. As predators and prey move together spatially and temporally throughout their habitat, individuals must make decisions to increase their fitness. Prey should move to improve their foraging rate and to avoid predators. Likewise, predators should be simultaneously moving to increase their own foraging (i.e., predation) success rate. In this way, one individual cannot "win" without another "losing." To make optimal movement decisions, individuals must accurately interpret their surroundings using available information.

Prey should attempt to estimate and respond to spatio- and temporal variation in predation risk. By estimating and responding perfectly to predation risk, prey can minimize the costs of reduced foraging while avoiding actual predation (Gilliam and Fraser, 1987) and avoid a phenotype-environment mismatch, where movement decisions do not correspond to actual environmental conditions (e.g., hiding from predators when no predators are nearby; Auld et al. 2010). However, to estimate predation risk, prey must have the ability to interpret environmental information.

A species may use visual, chemical, and/or mechanical cues to survey its environment. In aquatic systems, many prey organisms rely on chemical cues as environmental indicators (Kats and Dill, 1998). For predator-prey interactions, two chemical cues are important: predator-released "kairomones" and prey-released pheromones "alarm cues," the chemical cue components of a typical predation event. Kairomones consist of predator digestive enzymes and metabolites (i.e., predator digestive excretions), whereas alarm cues consist of secretions from damaged tissue (i.e., an injured prey) (Schoeppner and Relyea, 2005). During a predation event,

kairomones are produced and alarm cues are released while the prey is being consumed, especially in predators that consume prey outside of their body. After a predation event, the predator releases kairomones as they excrete digested prey tissue (Schoeppner and Relyea, 2009).

Kairomones and alarm cues can serve as a valuable source of information about the location of predators (i.e., kairomones only) or the location of recent successful predation events (i.e., kairomones and alarm cues combined) throughout an ecosystem. For example, studies have found that energetic antipredator behavior, specifically reductions in general activity levels, in tadpole prey increases with stronger chemical cue concentrations (i.e., combined predatory dragonfly kairomones and tadpole alarm cues) and increases asymptotically with length of chemical cue exposure (Fraker, 2008b; Ferrari and Chivers, 2009). However, chemical cues can be an unreliable information source to prey since they degrade with time and both fresh and aging chemical cues can be present in the environment at any given time (Turner and Montgomery, 2003; Peacor, 2006). Although recent cues provide reliable information about the location of a nearby predator, older cues may be located further away from where a mobile predator is currently located. Nevertheless, tadpoles were found to be unable to distinguish between fresh and up to 48-hour old chemical cue exposures (Fraker, 2008a). Furthermore, it is still unclear whether organisms can distinguish between the chemical cues produced during and after predation events. Thus, prey must incorporate uncertainty into predation risk estimates.

How predators and prey use available information to make movement decisions and how their space use differs after chemical cue exposure is not yet fully understood. Ecologists have used joint ideal-free-distribution models (IFD) to predict how predators and prey should move together within their environments (Lima and Dill, 1990; Sih, 1998). Joint IFD theory assumes that prey will distribute themselves among resource patches based on tradeoffs between predation risk and patch quality and that predators will attempt to match prey distributions. In turn, predators would be expected to match prey resource density within patches and prey would be expected to be evenly distributed throughout the ecosystem because the ratio of predation risk to resource density is the same across all patches. However, most joint IFD models assume that individuals have perfect information about surrounding resource patches, conspecifics, and predators, and that individuals have unlimited movement capability (Lima and Dill, 1990).

Additionally, these models simplify predator-prey behavioral interactions, given that the success of predators is a function of prey behavior and their contradictory movement objectives (Fraker and Luttbeg, 2012). Finally, joint IFD models do not consider that prey may be able to estimate spatial variation in predation risk that varies with habitat structure, termed the "landscape of fear" (Brown et al., 1999).

While the first generation of experiments testing predictions from joint IFD models found that tadpoles and larval dragonflies do qualitatively follow theoretical predictions, they also suggested that individuals may lack complete information about their environment and make movement decisions to manage fear and risk (Fraker and Luttbeg, 2012). Additional research that reflects complex interactions regarding predator-prey space use is needed. The first step to filling this research gap is to better understand how predators and prey move independently within an ecosystem.

Here, we address a key component of this research gap by asking two questions: How do predator and prey organisms use complex environmental information to estimate and respond to predation risk? And, how does predator and prey space use differ after varying chemical cue exposure? To begin to answer these questions, we measured space use within a model system consisting of predatory common green darner dragonfly (*Anax junius*) larvae and green frog (*Rana* [=*Lithobates*] *clamitans*) tadpoles. Both species were exposed to kairomones, alarm cues, or a combination of chemical cues.

We hypothesized that *R. clamitans* (hereafter referred to as tadpoles) would only display antipredator behavior (i.e., spatially move away from cue exposure to reduce encounters with predators) when reliable predictors of predation risk are present in the system. Prey species would be expected to consider both kairomones and alarm cues, as both are associated with predation events and both provide information about the predator and prey species involved in the predation event. Considering these chemical cues should help the tadpole prey forage optimally (i.e., minimize the ratio between predation risk and foraging). Since tadpoles do not appear capable of detecting the age of *Anax* kairomones (Fraker, 2008a), *Anax* space use may be regulated by prey-mediated non-consumptive impacts. Thus, we hypothesized that (1) tadpole

prey would respond more strongly when exposed to a combination of chemical cues and that (2) *Anax* would attempt to match prey distributions to increase its own rate of foraging.

Materials and Methods

Study System

Green frog egg masses and late-instar *Anax* larvae were collected during May 2016 from ponds at the Ohio Division of Wildlife's State Fish Hatchery located in Hebron, Ohio. Specimens were brought back to The Ohio State University's Aquatic Ecology Laboratory for housing and experiments (following IACUC protocol # 2016A00000028). The egg masses were cultured in 75 L wading pools filled with dechlorinated, aged tap water that was inoculated with plankton from native ponds. Once hatched, tadpoles were fed rabbit chow (Purina, St. Louis, MO, USA) *ad libitum*. *Anax* were housed in plastic containers filled with 400 mL of dechlorinated, aged tap water; water was changed weekly to maintain water quality. *Anax* were fed approximately 100 mg of live tadpoles three times per week.

Our experiments were conducted indoors in 75 L mesocosm pools filled with dechlorinated, aged tap water. The pools were located on the floor of a well-ventilated room with fluorescent lights set to a 14:10 light:dark schedule. Experimental pools were sectioned into four quadrants of equal size. Each quadrant contained one algal wafer disc (i.e., resource patch) and one completely submerged opaque plastic cup (i.e., habitat structure and refuge; Figure 1). Individuals were randomly selected for each experiment and placed into the experimental pools to acclimate overnight.

Chemical cue and control treatments (described below) were prepared fresh each experimental day. In each pool, one quadrant was randomly selected to receive the treatment effect. The chemical cue was added (preparation procedures described below) to the treatment quadrant slowly to minimize disturbance. The distribution of the subjects within the pools was recorded before chemical cue addition and every 30 min during a 2 to 4 hour period after chemical cue addition (Fraker, 2008b).

Experiment 1: Prey Space Use

This experiment measured the spatial response of three size classes of tadpoles to five combinations of chemical cues. Between 20-40 tadpoles from 1 of 3 size classes were randomly selected for use in this experiment. Densities in pools were lower than typically found in nature to avoid effects of intraspecific competition. The size classes were 50 mg (n = 14 rep.), 100-150 mg (n = 20 rep.), or 200 mg (n = 12 rep.) tadpoles. The five chemical cue treatments (and number of replicate pools used) were as follows: 1) caged, fed *Anax* (kairomones and alarm cues; n = 11); 2) caged, unfed *Anax* (kairomones only; n = 9); 3) tadpole-Triton cue (alarm cues only; n = 14); 4) Triton (control for alarm cue; n = 7.); and 5) water only(control; n = 5). The two caged *Anax* treatments were set up by placing a randomly selected *Anax* into the treatment quadrant's plastic cup. The cup opening was covered by fiberglass window screen so that the *Anax* could not escape and hunt the tadpoles; however, chemical cue could pass through. A caged *Anax* was either fed or not fed. If the *Anax* was to be fed, the *Anax* was provided a tadpole corresponding to the same size class as the observed tadpoles (50 mg, 100-150 mg, or 200 mg) at the start of the treatment, while in the cup. Thus, the caged *Anax* would produce kairomones and release tadpole alarm cues. The caged, unfed *Anax* would not be fed a tadpole and would produce kairomones only.

Amphibian tadpole pheromone is contained in tadpole skin cell vesicles and is only released via an active secretory process (Fraker et al. 2009). Thus, we combined live tadpoles corresponding to the same size class as the observed tadpoles (50 mg, 100-150 mg, or 200 mg) with a solution of 1% Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA) and 100 mL of de-ionized water to create an alarm cue only treatment (i.e., no kairomones produced). The detergent Triton X-100 solubilizes cell membranes and releases the pheromone into solution (Fraker et al., 2009). We sonicated the tadpoles, water, and Triton X-100 mixture for 30 s (Fisher Scientific Homogenizer Power Gen, Model 125, Fisher Scientific, Pittsburgh, PA, USA) to break up tadpole tissue cells and allow the Triton X-100 to cause the release of the pheromone. The solution was standardized with other 100-mL solutions to minimize confounding effects of varying tadpole cue production. A 100 mL solution containing only Triton X-100 and water was produced using the same procedure as a control for Triton X-100. A 100 mL water control was used as a control treatment.

Experiment 2: Predator Space Use

We conducted a second experiment to assess the spatial response of predatory *Anax* larvae to chemical cues. This experiment consisted of placing one randomly selected *Anax* into an experimental pool with different chemical cues. Only one *Anax* was used because predatory *Anax* spatially avoid one another (Fraker and Luttbeg, 2012). Since the purpose of this experiment was to determine how individual *Anax* spatially respond to chemical cue exposure, having more than one *Anax* could potentially bias movement decisions.

To assess whether satiation influenced *Anax* movement decisions, we created two *Anax* feeding treatments, both of which were crossed with six chemical cue treatments. *Anax* were either unfed (i.e., starved for 48 hours prior to use) or fed (i.e., fed tadpoles just prior to addition to a pool). The six chemical cue treatments (and number of replicate pools used) were as follows: 1) caged, fed *Anax* (visual, kairomones, and alarm cues; n = 12); 2) caged, unfed *Anax* (visual and kairomones; n = 12); 3) fed *Anax* cue (kairomones and alarm cues; n = 12); 4) starved *Anax* cue (kairomones only; n = 12 rep.); 5) tadpole-Triton cue (alarm cues only; n = 12); and 6) water only (control; n = 12). The caged *Anax* treatments were prepared using the same procedure as the tadpole space use experiments above. Non-visual cues were used to observe the effect of visual cues (i.e., a conspecific caged within the pool) versus non-visual cues (i.e., only the chemical cue from a conspecific) on *Anax* space use. The fed *Anax* cue was prepared by feeding housed, non-experimental *Anax* tadpoles. The water and chemical cue from each cup were then standardized with other fed *Anax* cue to minimize confounding effects of varying cue production. Each treatment pool received 100 mL of this cue. The starved *Anax* cue was prepared using the same procedure, except the *Anax* used to make the cue were starved for 48 hours prior to cue collection. The tadpole-Triton cue and water treatments were prepared in 100 mL volumes as described above for tadpole space use experiments.

Data Analysis

For each experiment, space use was calculated using the mean proportion of individuals in the treatment quadrant over each observation for each pool. Since the pools were divided into four quadrants, the expected proportion of individuals within the treatment quadrant was w 0.25. An avoidance space use would be less than 0.25, whereas an attracting space use would be greater than 0.25.

Two-way analysis of variance (ANOVA) was used to compare treatments in each experiment. Our models looked at the effect of the main factors (chemical cues, predator satiation) and their interaction. Tukey's honestly significant difference (Tukey's HSD) tests were used to identify treatment differences, when a main factor or the interaction term was found to be significant. All data met assumptions for normality using the Kolmogorov-Smirnov test (all $p > 0.05$) and homogenous variances using Levene's test. An alpha-value of 0.05 was used to indicate statistical significance. Statistical analyses were performed using JMP Pro 12.2 (SAS Institute Inc. 2015).

Results

Prey Space Use

Initial analyses showed that size class had no effect on tadpole space use ($p = 0.85$), but the interaction between size class and chemical cue had a significant effect on tadpole space use ($p = 0.026$; Table 1; Figure 2). Tukey's HSD tests suggested that there may be differences in space use between the 50 mg size class and the other two size classes, 100-150 mg and 200 mg (Figure 2). Since we expect spatial antipredator behavior to decrease with size class (Fraker, 2008b), we ran analyses with two size classes: 50 mg and 100-200 mg. These analyses found that neither size class ($p = 0.71$) nor the interaction between size class and treatment ($p = 0.50$) had a significant effect on space use, while chemical cue treatment had a significant effect on space use ($p = 0.0003$; Table 2; Figure 3). Therefore, in subsequent analyses we excluded the effect of size class, and pooled all individuals based on chemical cue treatment. We found a significant effect of chemical cue treatment on space use ($F = 7.492$; $df = 4$; $p < 0.0001$; Figure 4). Tadpole avoidance was strongest in the caged, fed *Anax* treatment ($\mu = 0.074 \pm 0.02$ SE). In contrast, the caged, unfed *Anax* ($\mu = 0.260 \pm 0.025$ SE), tadpole-Triton ($\mu = 0.175 \pm 0.026$ SE), and Triton control ($\mu = 0.229 \pm 0.029$ SE) treatments did not differ from the water control ($\mu = 0.215 \pm 0.027$ SE). Thus, tadpoles only responded to cue exposure from the caged, fed *Anax* treatment, which contained both kairomones and alarm cues.

Predator Space Use

Anax space use did not differ among any of the treatments or satiation levels (Table 3). Mean use of the treatment quadrant ranged from 0.21 ± SE to 0.33 ± SE (Table 3; Figure 5). Comparisons of the effects of *Anax* condition (fed, unfed) and treatment are shown in Figure 5.

Discussion

Our results show that tadpoles react with spatial antipredator behavior only when exposed to both *Anax* kairomones and conspecific alarm cues (i.e., a typical predation event). Tadpoles did not display spatial antipredator behavior in response to either kairomones or alarm cues alone. These results suggest that tadpoles maximize growth by only responding to specific chemical cues, thus minimizing potentially costly antipredator behavior. In contrast, *Anax* did not spatially avoid or were not spatially attracted to either *Anax* or tadpole cue, suggesting that, *Anax* move randomly in response to chemical cue exposure and satiation status. Together, these results suggest that these species are concurrently managing fear and risk through their spatial behavior.

We found little difference between tadpole size classes and space use. This result was surprising because previous research found that larger tadpoles exhibit shorter periods of antipredator behavior than smaller tadpoles (Fraker, 2008b). A proposed explanation for this result is that larger tadpoles respond less strongly because they experience decreasing vulnerability to predation as they increase in size (Eklov and Werner, 2000) and thus perceive less risk (Fraker, 2008b). We are uncertain as to why we did not find a similar finding in our experiment. Our results suggested a small difference between 50 mg and the larger size classes of tadpoles, but this was not statistically significant. Since the relationship between tadpole size and space use was inconsistent, we combined treatment levels from all size classes to obtain higher statistical power for our chemical cue exposure treatments.

Predicting prey behavior requires an understanding of the factors that affect perceived risk and the subsequent prey response. Gilliam and Fraser (1987) suggested that prey should minimize its predator encounter rate and visibility while maximizing foraging. Our research supported this prediction, which has also been supported in a broad range of systems (Lima, 1998). We found that tadpole space use significantly varied from control levels, but only when exposed to both *Anax* kairomones and tadpole alarm cues. Neither *Anax* kairomones nor tadpole

alarm cues alone were enough to elicit antipredator behavior. This finding suggests that tadpoles have adapted to selectively respond to some information and ignore other information. A possible explanation is that the scent of a predator does not suggest a high enough risk unless evidence also exists to indicate that the predator is successfully foraging.

Since tadpoles respond spatially with antipredator behavior when exposed to both kairomones and alarm cues, we would expect that *Anax* would also move spatially away from these cues. However, we found that *Anax* moved randomly in response to chemical cues used in our experiment. If tadpoles are actively avoiding predators by moving away from predation events, but the predators are randomly distributed throughout the environment, then the tadpoles may not be reducing their average likelihood of encountering a predator. In this way, random movement by *Anax* may help spatially distribute the location of chemical cues, and hence, behaviorally manage prey fear (Mitchell and Lima, 2002). *Anax* predatory behavior has been shown to differ from IFD predictions elsewhere. For example, Fraker and Luttbeg (2012) found that paired *Anax* in mesocosm pools made similar movement decisions, maintained greater distances from each other than predicted, and moved shorter distances than predicted. Together, these results suggest that *Anax* move randomly to manage the perceived risk in a given area.

Optimal antipredator behavior is essential for prey organisms. Evolutionary fitness ultimately depends on a minimum positive growth rate (Gilliam and Fraser, 1987). In prey species that respond to perceived predation risk with a reduction in foraging, excessive antipredator behavior may result in insufficient energy intake. In larval amphibians, reduced growth is associated with several negative effects (Relyea, 2001). First, tadpole morphology is plastic in the presence of predators. In tadpoles exposed to predators, the tadpoles develop deeper tails, deeper tail muscles, and shorter trunks. This plasticity appears to help provide resistance to predation; however, this development comes at the cost of slower growth and development. Coupled with the effect of decreased foraging activity and increased refuge use, there can be a decrease in fitness (Relyea, 2007). Smaller tadpoles have increased susceptibility to pool drying and vulnerability to desiccation. Many amphibians have evolved the ability to adjust the amount of time they spend in the larval stage based on perceived environmental risk. Since the ability to metamorphose is positively related to size, smaller tadpoles may not metamorphose before winter and thus overwinter as tadpoles. Once smaller tadpoles do metamorphose, they experience

lower survivorship than larger froglets. Thus, the non-consumptive effect of predators in this system can have strong potential to shape prey dynamics.

The individual movement decisions of predators and prey can shape how the two species move together both in time and space. Future studies should continue to explore what environmental information predator and prey species use to make movement decisions. A more mechanistic understanding of how species estimate and respond to risk and environmental variability is needed. The reliability of environmental information and how this imperfect information shapes game dynamics should also be investigated. Further research needs to consider experiments that acknowledge that organisms do not have perfect knowledge of their surrounding environment and that individuals may not have unlimited movement capabilities to better replicate natural conditions.

Limitations

Our experiments controlled for differences in multiple sources of environmental information that varies spatially and temporally so that we could determine the basic mechanisms for making movement decisions. In turn, our ability to generalize our findings to natural systems is limited in some ways. First, we minimized habitat structure heterogeneity. The experimental arenas were constructed with minimal differences in habitat structure among treatments. Since hiding from predators is a typical antipredator behavior, habitats with more available refuge and structure could be expected to influence movement decisions. Second, we did not examine how temporal variation in predation risk impacts space use. Experimental arenas where chemical cues are randomly produced throughout the experimental period are a more realistic depiction of natural systems. Third, our study did not consider heterospecific predator and prey chemical cues. Previous research has shown that tadpoles are capable of distinguishing between heterospecific chemical cues (Fraker, 2009a; Schoeppner and Relyea, 2005). The antipredator response is related to the risk posed by each predator to the specific prey species in question and if the prey consumed was a conspecific or a different prey species. By only using one predator – prey relationship, we are excluding other ecological interactions that shape game dynamics. Fourth, the chemical cues examined here may not be wholly representative of what these species interpret to make movement decisions. Although we found that *Anax* move

randomly in response to these cues, they move non-randomly in response to prey movement, different prey species, etc. Further research is needed to determine if *Anax* move non-randomly in response to other environmental information. Finally, this study is limited in that it does not examine predator and prey species moving together spatially. As previously discussed, the consumptive effects of predators are important ecologically and need to be considered. However, despite these limitations, our results are still meaningful in that they help to provide the baseline mechanism for predator and prey movement decisions.

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Tables

Table 1. ANOVA results from an experiment that explored the effect of tadpole size, chemical cue treatment, and their interaction on tadpole space use. An asterisk (*) denotes significant effects.

Treatment	DF	F Ratio	Probability > F
Tadpole Size	2	0.03	0.972
Chemical Cue	4	8.64	< 0.0001*
Size*Chemical Cue	8	2.54	0.0259*

Table 2. ANOVA results from an experiment that explored the effect of tadpole size, chemical cue treatment, and their interaction on tadpole space use. Based on results from a previous analysis (Table 1), the size classes were pooled into 50 mg and 100-200 mg classes. An asterisk (*) denotes significant effects.

Treatment	DF	F Ratio	Probability > F
Tadpole Size	1	0.14	0.713
Chemical Cue	4	6.62	0.0003*
Size*Chemical Cue	4	0.85	0.499

Table 3. ANOVA results from an experiment that explored the effect of predator condition (fed, unfed), chemical cue treatment, and their interaction on predator space use. An asterisk (*) denotes significant effects.

Treatment	DF	F Ratio	Probability > F
Anax Condition	1	0.006	0.939
Chemical Cue	5	0.102	0.991
Condition*Chemical Cue	5	0.047	0.999

Table 4. Summary statistics for the *Anax* space use experiment. The effect of *Anax* condition is shown and the interaction between condition and treatment is detailed.

Treatment	**Mean**	**Standard Error (SE)**
Fed	0.264	0.0605
Caged, Fed *Anax*	0.292	0.1873
Caged, Unfed *Anax*	0.250	0.1581
Fed *Anax* Cue	0.292	0.1873
Starved *Anax* Cue	0.208	0.1003
Tadpole-Triton	0.292	0.1502
Water Control	0.250	0.1581
Starved	0.271	0.0601
Caged, Fed *Anax*	0.333	0.1787
Caged, Unfed *Anax*	0.333	0.1394
Fed *Anax* Cue	0.250	0.1708
Starved *Anax* Cue	0.208	0.1357
Tadpole-Triton	0.250	0.1581
Water Control	0.250	0.1581

Figures

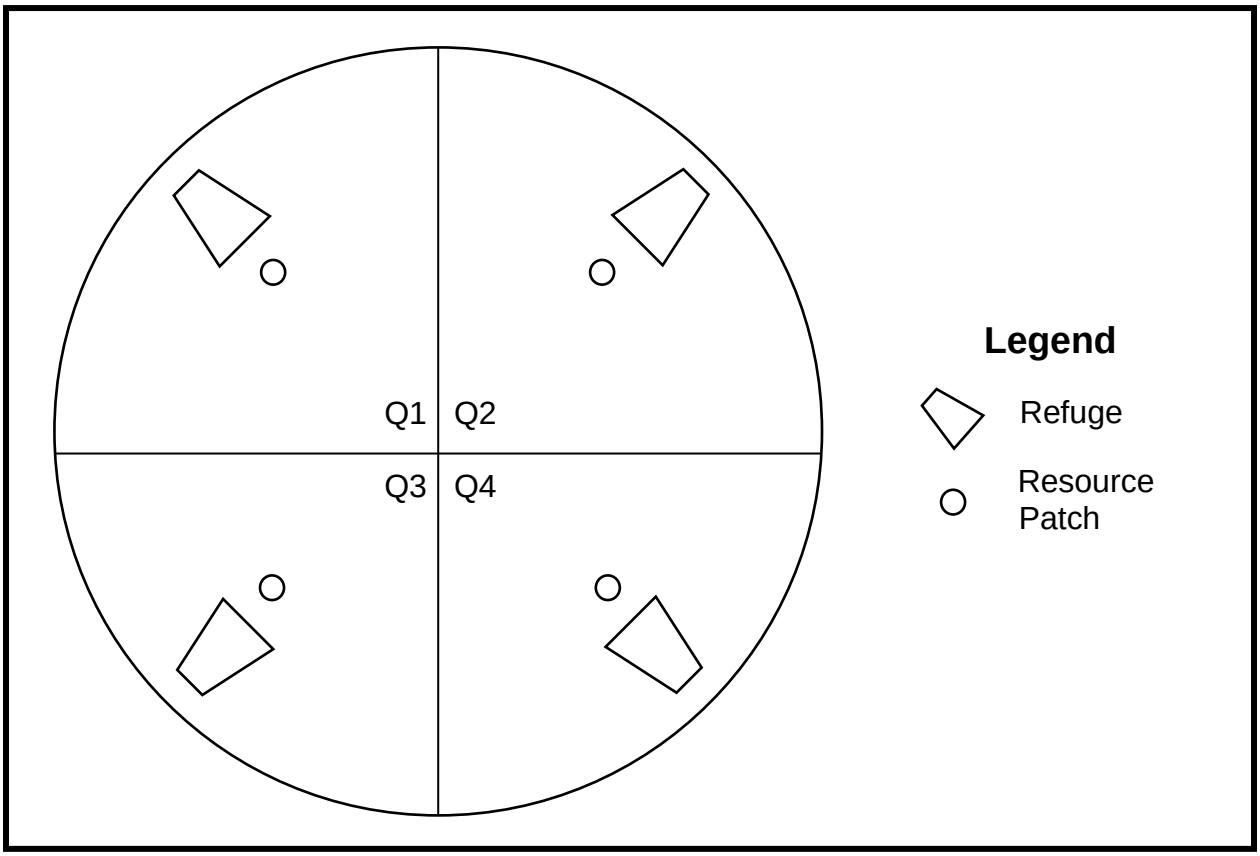

Figure 1. Mesocosm setup for quantifying space use. Experimental mesocosms consisted of a 75-L pool separated into four equal quadrants (Q1, Q2, Q3, or Q4). Each quadrant contained an opaque plastic cup refuge and an algal disc resource patch. Q1, which was randomly determined for each replicate, received the chemical cue treatment.

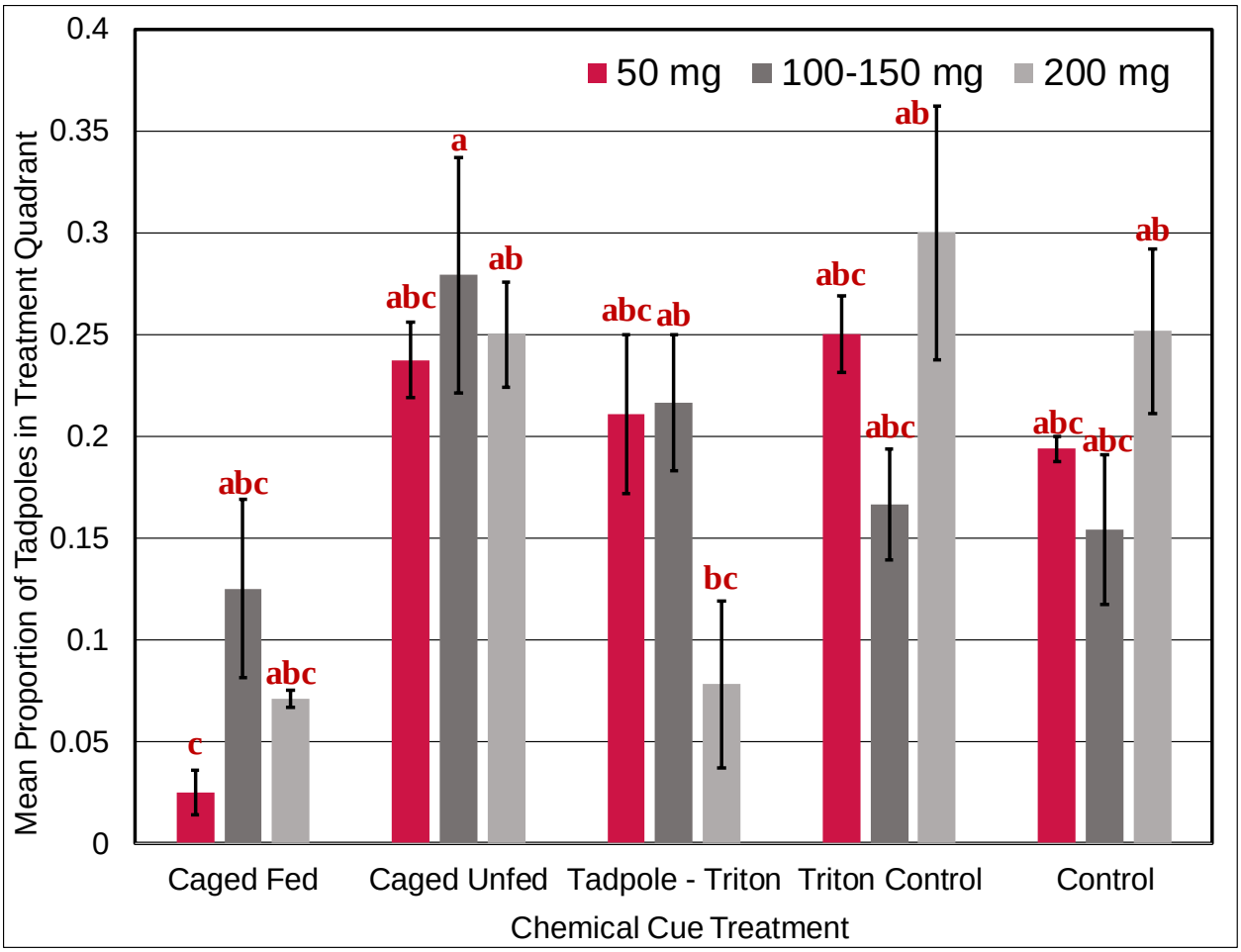

Figure 2. Space use by tadpoles exposed to different combinations of chemical cues crossed by size class (50 mg, 100-150 mg, 200 mg). Mean proportion is displayed ± 1 SE. A proportion equal to 0.25 indicates that tadpoles were randomly distributed. An avoidance response is signified by a proportion less than 0.25 and an attraction response is signified by a proportion greater than 0.25. Post hoc analysis using Tukey's HSD test is displayed using letters above SE bars. Means with letters in common do not vary significantly.

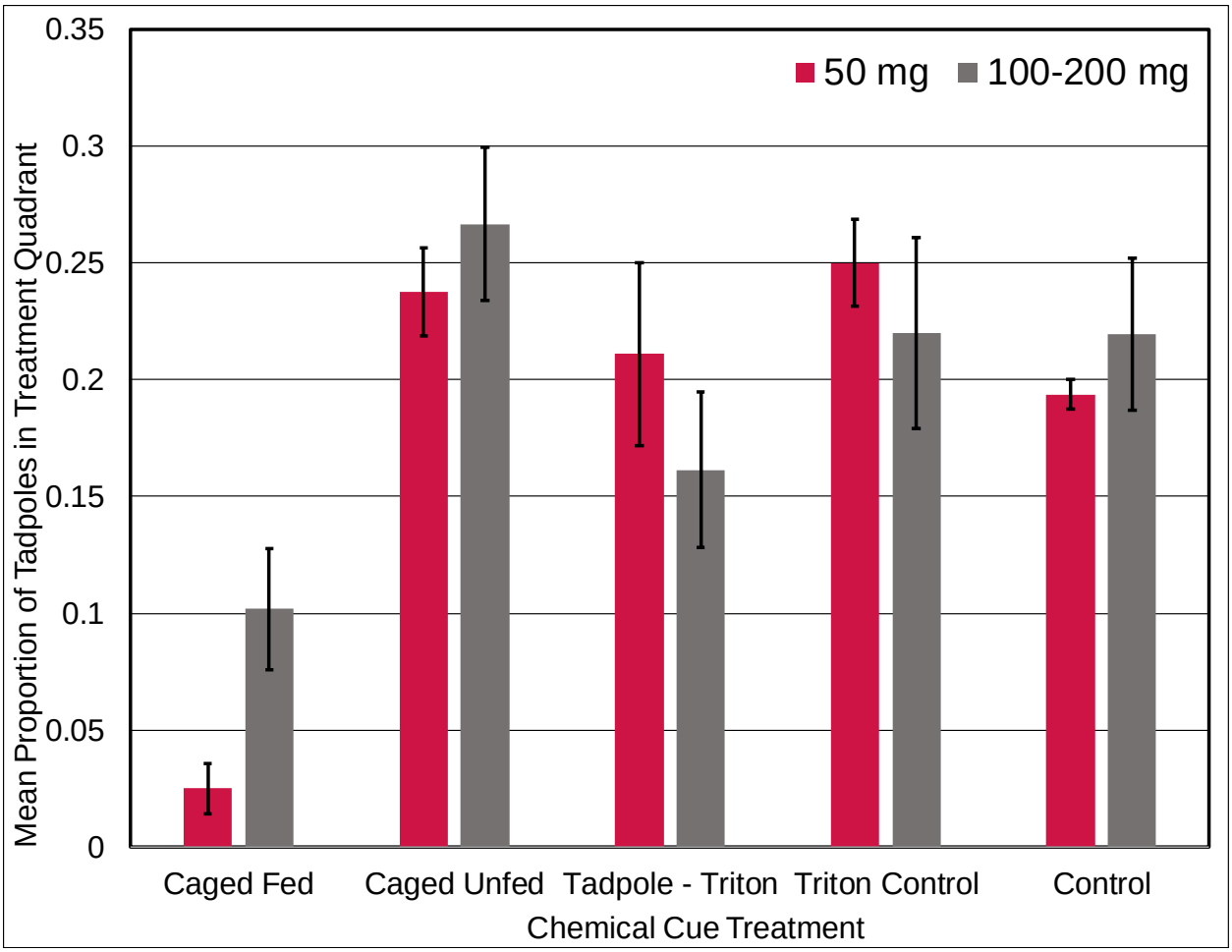

Figure 3. Space use by tadpoles exposed to different combinations of chemical cues crossed by size class (50 mg, 100-200 mg). Mean proportion is displayed ± 1 SE. A proportion equal to 0.25 indicates that tadpoles were randomly distributed. An avoidance response is signified by a proportion less than 0.25 and an attraction response is signified by a proportion greater than 0.25.

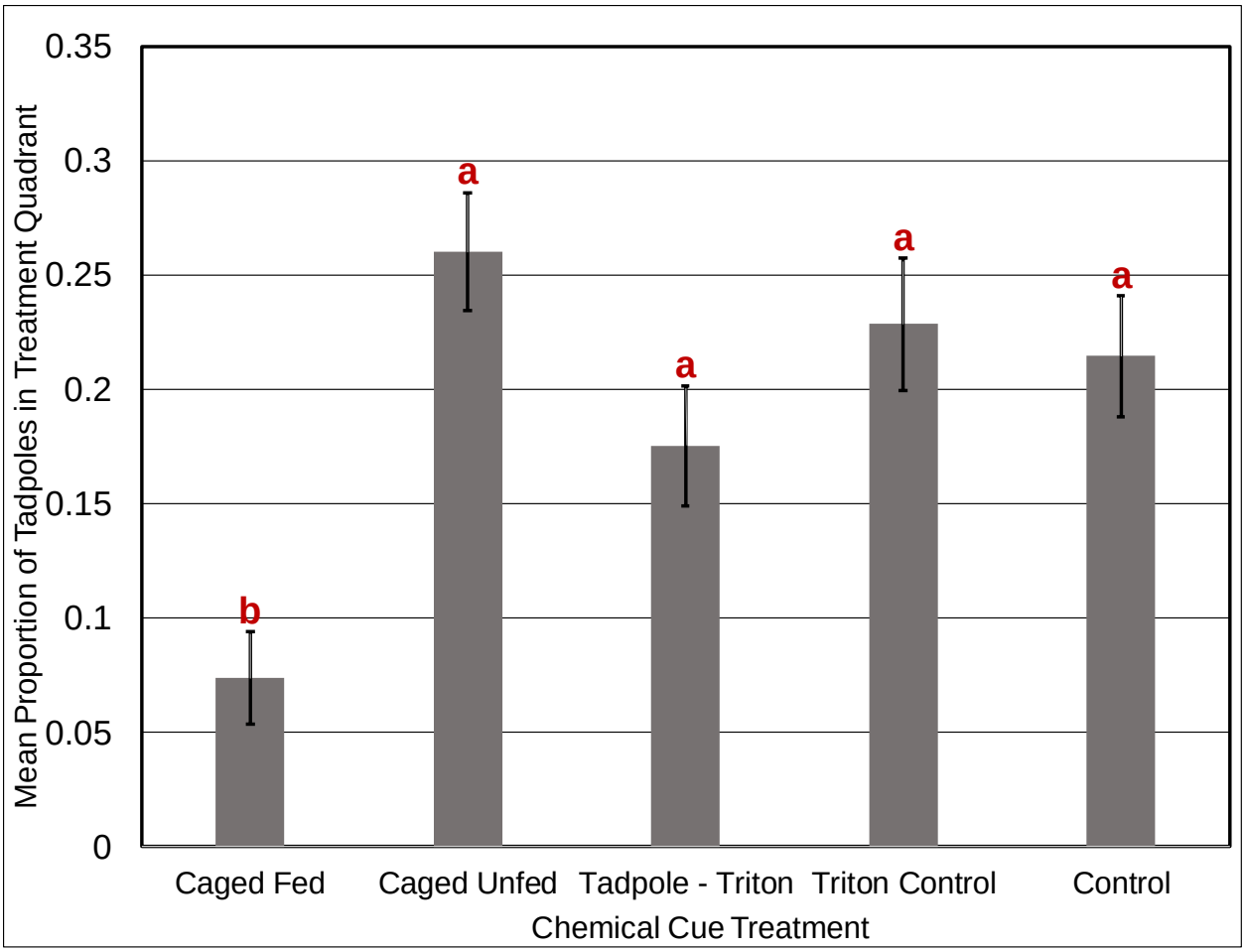

Figure 4. Space use by tadpoles exposed to different combinations of chemical cues. Mean proportion is displayed ± 1 SE. A proportion equal to 0.25 indicates that tadpoles were randomly distributed. An avoidance response is signified by a proportion less than 0.25 and an attraction response is signified by a proportion greater than 0.25. Since the effect of size class on chemical cue was found not to be significant, size class was excluded from analysis. The effect of chemical cue was found to be significant ($p < 0.0001$). Post hoc analysis using Tukey's HSD test is displayed using letters above SE bars. Means with letters in common do not vary significantly.

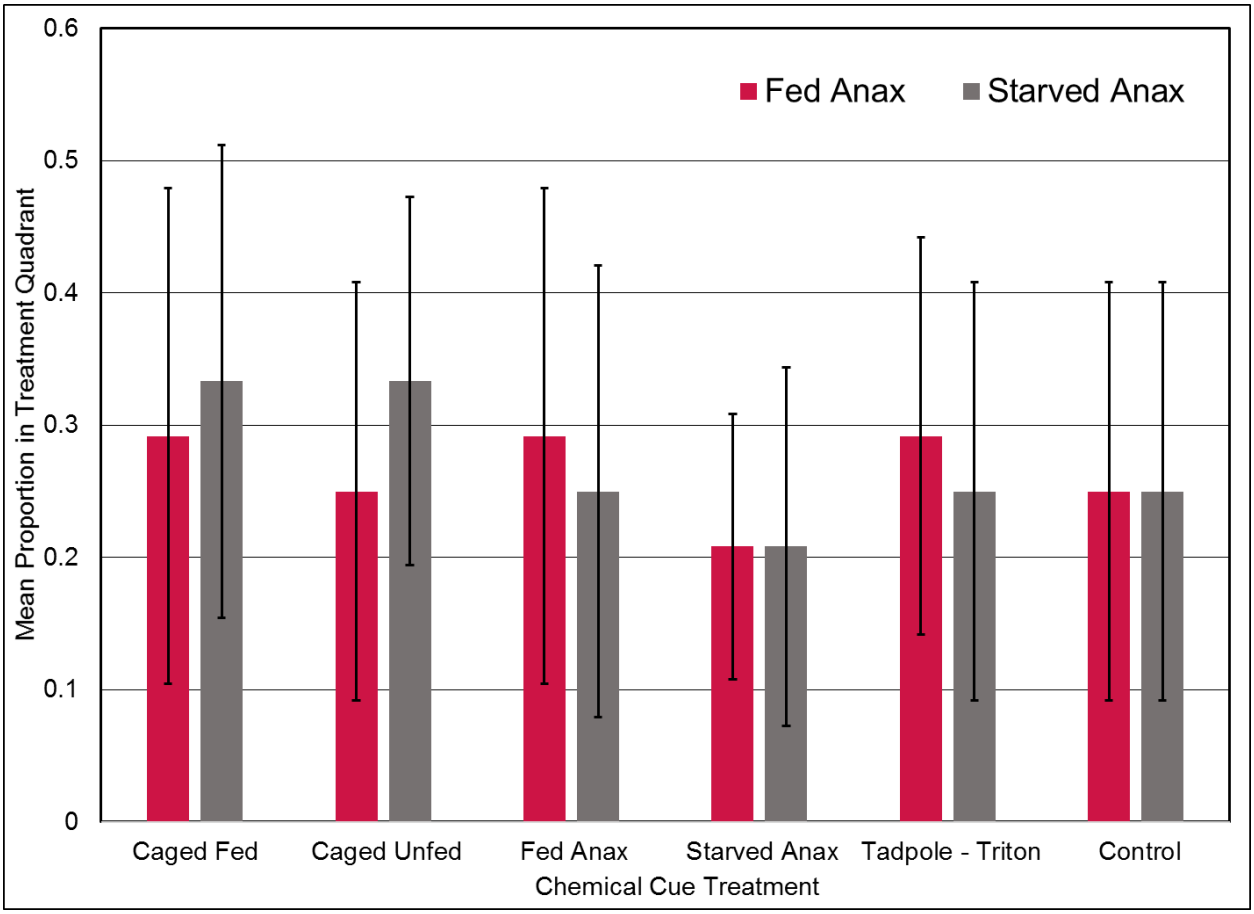

Figure 5. Space use by *Anax* exposed to different combinations of chemical cues crossed by satiation level (fed, unfed). The mean proportion for each treatment is displayed ± 1 SE. A proportion equal to 0.25 indicates that tadpoles were randomly distributed. An avoidance response is signified by a proportion less than 0.25 and an attraction response is signified by a proportion greater than 0.25.